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Short communication

Collection of *Trifolium* sp. and other forage legumes in Bulgaria

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Abstract

Joint collecting expeditions to improve the genetic diversity of *Trifolium* species in the U.S. National Plant Germplasm System (NPGS) were carried out in Bulgaria by scientists of the USDA-ARS, University of Florida, Texas A&M University, and Institute of Introduction and Plant Genetic Resources (IIPGR), Bulgaria, on 14–27 July 1990 and 30 July – 13 August 1993. The objectives were to collect seed of *T. vesiculosum* Savi and other annual clovers in southeastern Bulgaria in 1990 and seed of perennial *Trifolium* species in southwestern Bulgaria in 1993. A total of 246 collections were made of 50 *Trifolium* species (38 annual and 12 perennial) and 25 collections of 17 other forage legumes. Collections were made at 90 sites in 13 of the 20 floristic regions in Bulgaria. The genetic diversity of *T. vesiculosum* in the NPGS was increased by the addition of 16 accessions. Four *Trifolium* sp. were added to the NPGS. Land utilization for intensive grazing near villages and small plot farming in river valleys greatly increased between 1990 and 1993. Genetic erosion of the extensive *Trifolium* resources within Bulgaria may occur as these practices increase.

Abbreviations: GPS – Global Positioning System; GRIN – Germplasm Resources Information Network; IIPGR – Institute of Introduction and Plant Genetic Resources; NPGS – U.S. National Plant Germplasm System.

Introduction

The genus *Trifolium* is quite extensive, containing between 233 and 245 species (Mundell & Taylor, 1996). The most complete treatment of the genus (Zohary & Heller, 1984) lists 237 species, though a number of species native to the western United States (Martin, 1943) are not included. Germplasm collection efforts worldwide have concentrated on 10-20 agronomically important species with little effort made to preserve the vast array of species within the genus. In 1977, Taylor et al. reported that the United States National Plant Germplasm System (NPGS) had seeds of only 85 *Trifolium* species, 70 annual and 15 perennial, available for distribution. Since that time, scientists in the Clover and Special Purpose Legume Crop Germplasm Committee have conducted a number of collection expeditions in eastern Europe and Mediterranean areas (Smith et al., 1978; Taylor & Rumbaugh, 1986; Taylor & Smith, 1990) through the support of the Plant Exploration Office (more recently the Plant Exchange Office), Agricultural Research Service, United States Department of Agriculture.

Bulgaria contains at least 28% of the known *Trifolium* species. Previous reports (Kozuharov, 1976; Petrova & Kozuharov, 1982a,b; Zohary & Heller, 1984) indicate that 67 (51 annual and 16 perennial) of the 237 *Trifolium* species have been observed in Bulgaria. To improve the genetic diversity of *Trifolium*

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species in the NPGS, joint collecting expeditions were organized to collect *T. vesiculosum* and other annual species from low elevations in southeastern Bulgaria in 1990 and perennial *Trifolium* species from mountainous areas of southwestern Bulgaria in 1993. Additionally, Dr. Guteva of the Institute of Introduction and Plant Genetic Resources (IIPGR), Sadovo, Bulgaria earlier collected two accessions that are included with the 1990 totals, and 12 accessions during 1991-1992 that are included with the 1993 totals.

Collection procedure

The expeditions were conducted from 14–27 July 1990 and 30 July to 13 August 1993. The dates of collection were based on previous experience by IIPGR scientists which suggested annual *Trifolium* species would mature in July and perennial species in August. In 1990, the plants matured earlier than expected and all annual *Trifolium* species collected were completely mature and dry. The plants had been well preserved by the dry climate, and collection and identification was possible at most sites. In 1993, the collection dates were too early in some cases as plants of perennial species such as *T. medium* L. and *T. pannonicum* Jacq. were not fully mature. However, hay harvesting was being conducted at a number of high elevation meadows throughout southwestern Bulgaria during August 1993. Delay of the expedition by a week or two would have resulted in fewer sites at which to collect.

During the 1993 expedition, latitude and longitude were measured using a Global Positioning System (GPS) provided by the Plant Exploration Office. In 1990, a GPS was not available and latitude and longitude were estimated to the nearest degree and minute by road map location. Altitude was measured using an altimeter during both expeditions since the altitude readings given by the GPS in 1993 were occasionally inaccurate. In 1993, soil samples were taken at most sites. The pH was then determined by a portable pH meter. An inexpensive soil texture kit was used to determine the relative amounts of sand, silt, and clay in each sample. No soil samples were taken in 1990.

Seed collections were made at sites that were open hillsides, native meadows, or gullies or forest openings often under oak or other deciduous trees. No collections were made at sites known to have been reseeded or under active tillage management. Every effort was made to collect a great diversity of *Trifolium* species from a wide range of habitats rather than making extensive collections of any particular species (other than *T. vesiculosum*).

All seed collections made from both expeditions have been deposited in the NPGS. Additional information on individual accessions and collection sites may be obtained from the NPGS Germplasm Resources Information Network (GRIN). Germplasm deposited in the NPGS is available in small quantities for researchers worldwide. Collections made during these expeditions were shared with IIPGR scientists as desired for the IIPGR collection.

Collection sites

Collections were made in 13 of the 20 floristic regions of Bulgaria as described by Kozuharov (1976) during the two expeditions (Figure 1). These sites encompassed the southern half of Bulgaria ranging from the Black Sea coast in the east to the Western Frontier mountain region in the west. The majority of the collections were made in the Tundza hills, Tracian plain, and Strandza, Rhodope, Rila, and Pirin mountains.

1990 Expedition: Participants traveled over 2000 km throughout southeastern Bulgaria in 1990. Elevation of collection ranged from 30 m [*Medicago orbicularus* (L.) Bartl.] near the Black Sea coast to 1150 m (*T. alpestre* L., *T. ochroleucum* Huds., and *Lotus corniculatus* L.) west of Batak in the Rhodope mountains. Most of the collection sites were at low elevations with 39% of the collections made at elevations less than 300 m and 65% made at elevations less than 500 m. Soil texture was estimated by visual observation in 1990. Of 30 sites surveyed, the soils were predominantly rocky (37%), clay loam (27%), or clay (20%).

1993 Expedition: Participants traveled over 4000 km throughout southwestern Bulgaria in 1993. Elevation of collection ranged from 140 m (*T. repens* L.) at Plovdiv to 1790 m (*T. pratense* L. and *T. aureum* Poll.) in the Pirin mountains near Bansko. Most of the collection sites were at higher elevations than the 1990 expedition with 45% of the collections made at elevations greater than 1000 m and 75% made at elevations greater than 500 m. Soil pH and texture were determined from soil samples taken at 28 sites during the 1993 expedition. Soil pH ranged from 5.4 in the Pirin mountains near Bansko to 8.4 in the central Rhodope mountains near Pamporovo with a median value of

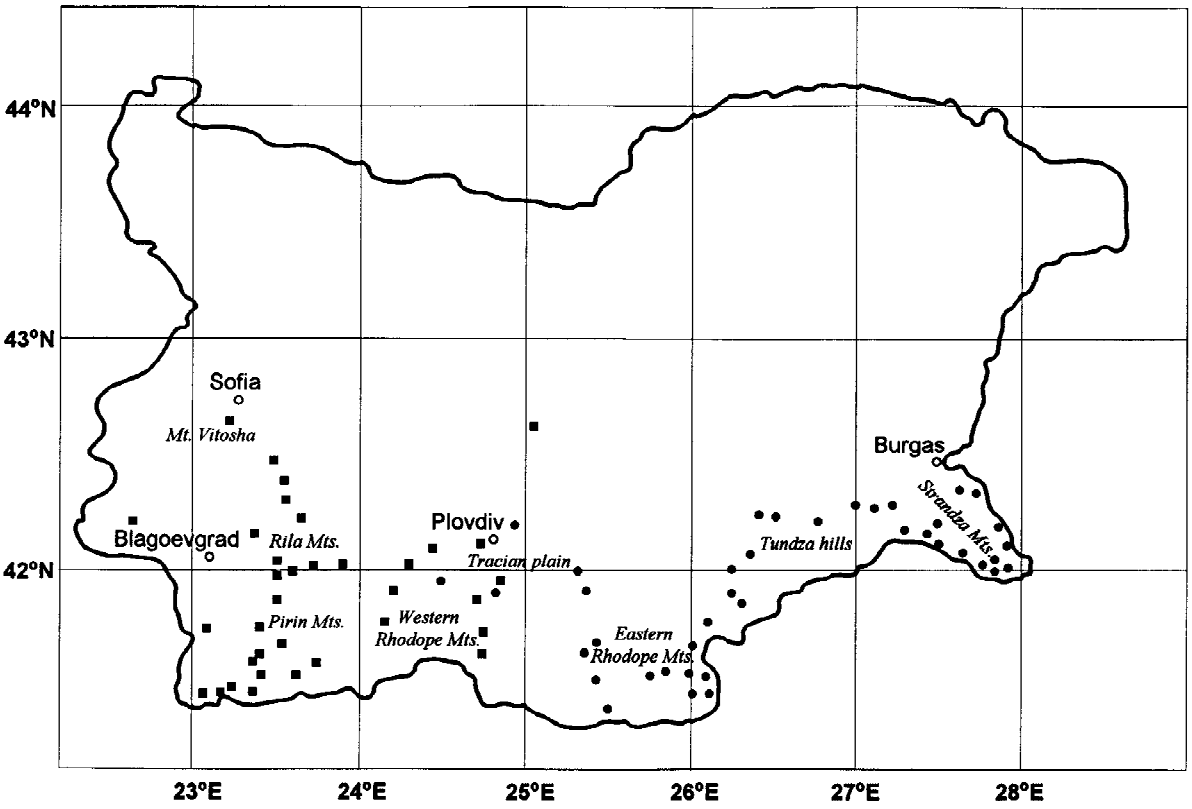

Figure 1. Collection sites in Bulgaria in 1990 (●) and 1993 (■).

6.8. Almost 43% of the soils had a pH value ranging from 6 to 7 while 25% had a pH value of less than 6. Soil texture ranged from a clay-clay loam in the Rhodope mountains near Pamporovo to a sand in the Pirin mountains near Jane Sandanski. The soils were predominantly sandy loam (50%) or sandy clay loam (25%).

Trifolium species collected

During the 1990 expedition, a total of 143 collections were made of 35 *Trifolium* species and one species not yet identified (Table 1). Nine collections were made of six other forage legume species (Table 2). In 1993, a total of 103 collections of 42 *Trifolium* species were made (Table 1) and 16 collections were made of 13 other forage legumes (Table 2). Most collections were made of *T. vesiculosum* Savi (16), *T. alpestre* (16), and *T. hirtum* All. (14).

Strandza mountains: A total of 25 collections of 18 *Trifolium* species were made at 13 sites in the Strandza mountains (Table 3). Species collected in these expeditions that were not reported to be in the Strandza mountain floristic region (Kozuharov, 1976) were *T. diffusum* Ehrh. and *T. strictum* L. The only accessions of *T. lappaceum* L., *T. ligusticum* Balb. ex Loisel., and *T. suffocatum* L. were obtained in this region.

Tundza hills: A total of 30 collections of 17 *Trifolium* species were made at 10 sites in the Tundza hills region (Table 3). *T. affine* C. Presl, *T. heldreichianum* (Gib. & Belli) Hausskn., and *T. trichopterum* Pancic were collected in this region, but not previously reported there (Kozuharov, 1976). Also, *T. alpestre* was observed outside of its normal habit in Bulgaria (600-2000 m; Kozuharov, 1976) at an altitude of 280 m. The only collection of *T. affine* was obtained in this region.

Tracian plain: A total of 20 collections of 13 *Trifolium* species were made at 6 sites in the Tracian plain (Table 3). One of the collections still has not been identified to species. The only species not previously

Table 1. Summary of *Trifolium* species collected in Bulgaria

	Growth habit	Number of collections	
		1990	1993
Trifolium affine C. Presl	A[a]	-	1
Trifolium alpestre L.	P	9	7
Trifolium angustifolium L.	A	3	2
Trifolium arvense L.	A	1	2
Trifolium aureum Poll.	A	-	6
Trifolium badium Schreb.	P	-	1
Trifolium bocconei var. *tenuifolium* (Ten.) Griseb.	A	3	1
Trifolium campestre Schreb.	A	2	1
Trifolium caucasicum Tausch	P	-	1
Trifolium cherleri L.	A	4	-
Trifolium constantinopolitanum Ser.	A	1	-
Trifolium diffusum Ehrh.	A	6	4
Trifolium dubium Sibth.	A	-	2
Trifolium echinatum M.B.	A	3	1
Trifolium fragiferum L.	P	5	2
Trifolium glomeratum L.	A	2	2
Trifolium grandiflorum Schreb.	A	1	2
Trifolium haussknechtii Boiss.	A	-	1
Trifolium heldreichianum (Gib. & Belli) Hausskn.	P	7	1
Trifolium hirtum All.	A	11	3
Trifolium hybridum L.	P	8	4
Trifolium incarnatum var. *molinerii* (Balb. ex Hornem.) Ser.	A	-	2
Trifolium lappaceum L.	A	-	1
Trifolium leucanthum M.B.	A	1	2
Trifolium ligusticum Balb. ex Loisel.	A	-	1
Trifolium medium L.	P	1	7
Trifolium michelianum Savi	A	3	-
Trifolium montanum L.	P	1	3
Trifolium nigrescens Viv.	A	3	2
Trifolium ochroleucum Huds.	P	8	3
Trifolium pallidum Waldst. & Kit.	A	5	-
Trifolium pannonicum Jacq.	P	-	5
Trifolium patens Schreb.	A	2	3
Trifolium phleoides Pourr. ex Willd.	A	-	2
Trifolium pratense L.	P	5	3
Trifolium purpureum Loisel.	A	5	2
Trifolium repens L.	P	6	4
Trifolium resupinatum L.	A	1	-
Trifolium retusum L.	A	3	-
Trifolium scabrum L.	A	6	2
Trifolium setiferum Boiss.	A	-	2
Trifolium spadiceum Vill.	A	-	6
Trifolium squamosum L.	A	-	1
Trifolium striatum L.	A	1	3
Trifolium strictum L.	A	5	2
Trifolium subterraneum L.	A	1	1
Trifolium suffocatum L.	A	-	1
Trifolium trichopterum Pancic	A	3	-
Trifolium velenovskyi Vandas	A	1	-
Trifolium vesiculosum Savi	A	15	1
Trifolium sp. (unknown)	-	1	-
Total		143	103

Table 1. (Continued)

[a] A=annual, P=perennial.

reported in the Tracian plain (Kozuharov, 1976) was *T. echinatum* M.B.

Rhodope mountains: The greatest diversity of *Trifolium* species was observed in the Rhodope mountain floristic region. Therefore, more time was spent and collections made in the Rhodope mountains than in any other region. A total of 107 collections of 37 *Trifolium* species were made at 30 sites in the eastern, central, and western Rhodope mountains (Table 3).

Table 2. Summary of forage legumes other than *Trifolium* sp. collected in Bulgaria

	Number of collections
Astragalus monspessulanus L.	1
Cytisus sp.	1
Hippocrepis unisiliquosa L.	1
Lotus corniculatus L.	3
Lupinus sp.	2
Medicago arabica (L.) Huds.	2
Medicago sativa ssp. *falcata* (L.) Arcang.	1
Medicago minima var. *brevispina* Benth.	2
Medicago minima (L.) Bartal.	1
Medicago orbicularus (L.) Bartal.	2
Medicago rhodopea Velen.	1
Melilotus officinalis Lam.	1
Onobrychis sp.	2
Onobrychis viciifolia Scop.	1
Securigera varia (L.) Lassen	2
Vicia cassubica L.	1
Vicia ervilia (L.) Willd.	1
Total	25

Table 3. Summary of altitude, soil data, and annual and perennial *Trifolium* species collected by floristic regions of Bulgaria

	Strandza mts.			Tundza hills			Tracian plain			Rhodope mts.			Rila mts.			Pirin mts.		
	min	max	med[a]	min	max	med	min	max	med	min	max	med	min	max	med	min	max	med
Altitude	50	455	250	110	400	265	140	400	270	150	1570	710	870	1560	975	645	1790	905
Soil pH	6.0	8.0	7.0	-	-	-	-	8.0	-	5.8	8.4	6.8	5.8	6.6	6.3	5.4	7.5	6.8
Soil texture	clay loam			clay loam			sandy loam			sandy loam			sandy loam			sandy loam		
	loam			clay			clay loam			sandy clay loam			loam			sandy clay loam		
	clay						clay			clay loam						sand		
No. of *Trifolium* spp. collected																		
Annual	12			11			12			27			6			13		
Perennial	6			6			1			10			4			3		

[a] min = minimum, max = maximum, med = median.

Species collected during these expeditions that were not previously reported in the Rhodope mountains (Kozuharov, 1976) were *T. aureum*, *T. echinatum*, *T. michelianum* Savi, *T. nigrescens* Viv., and *T. phleoides* Pourr. ex Willd. Three species, *T. glomeratum* L. and two collections each of *T. bocconei* var. *tenuifolium* (Ten.) Griseb. and *T. strictum*, were collected at higher altitudes (140–340 m higher) than normally observed (Kozuharov, 1976). Two species, *T. alpestre* and *T. badium* Schreb., were collected at a lower altitude (150–195 m lower) than normally observed.

Rila mountains: A total of 17 collections of 10 *Trifolium* species were made at 9 sites in the northern and southern Rila mountains (Table 3). Most of the collections were made in the southern part of the Rila mountains, as few species were noted at several sites in the northern Rila mountains. The only species collected in the Rila mountains that was not previously reported (Kozuharov, 1976) was *T. aureum*.

Pirin mountains: A total of 17 collections of 16 *Trifolium* species were made at 7 sites in the Pirin mountains (Table 3). *T. aureum*, *T. diffusum*, *T. glomeratum*, *T. haussknechtii* Boiss., *T. leucanthum* M.B., *T. phleoides*, and *T. squamosum* L. were all collected in the Pirin mountains, though they had not previously been reported in this floristic region (Kozuharov, 1976). *Trifolium haussknechtii* had not been reported previously in Bulgaria. *Trifolium glomeratum*, *T. leucanthum*, *T. phleoides*, and *T. squamosum* were collected at 150–370 m higher elevation than normally observed (Kozuharov, 1976).

Other floristic regions: A total of 29 collections of 24 *Trifolium* species were made at 15 sites in the Black Sea coast, Vitosha mountain, Western Frontier mountains, Sredna Gora mountains, Struma valley, Belasica mountain, and Mesta valley floristic regions. Species that were collected outside their known area of distribution (Kozuharov, 1976) were *T. bocconei* var. *tenuifolium* and *T. caucasicum* Tausch in the Belasica mountain region; *T. echinatum* in the Struma valley; *T. glomeratum*, *T. nigrescens*, and *T. purpureum* Loisel. in the Western Frontier mountains, and *T. setiferum* Boiss. in the Sredna Gora mountains.

Discussion

The objectives of both expeditions were achieved by the collection of 50 different *Trifolium* species (12 perennial and 38 annual) with one collection still unidentified. The genetic diversity of *T. vesiculosum* in the NPGS was increased by the addition of 16 accessions as well as two accessions of the close relative *T. setiferum*. Four *Trifolium* species added to the NPGS from these expeditions were *T. incarnatum* var. *molinerii* (Balb. ex Hornem.) Ser. (2 accessions), *T. setiferum* (2 accessions), *T. trichopterum* (3 accessions), and *T. velenovskyi* Vandas (1 accession). Numerous species that were collected had very few accessions in the NPGS previously. For example, the NPGS collection of *T. spadiceum* Vill. went from 2 to 8 accessions and *T. strictum* went from 4 to 11 accessions following these expeditions.

Certain collections had plants with traits that were previously unknown or uncommon for that species. A *T. heldreichianum* collection made alongside a trail

near Pamporovo in the Rhodope mountains had a rhizomatous rather than non-rhizomatous root system. A *T. patens* Schreb. collection made near Batak in the Rhodope mountains had multifoliate (4–5 leaflets) rather than trifoliate leaves. Collections of *T. patens* from the Rhodope mountains and *T. dubium* Sibth. from the Pirin mountains were late maturing in comparison to other accessions from the NPGS when grown in the greenhouse.

Some areas that had been reported to contain *Trifolium* species in historical references (Kozuharov, 1976; Zohary & Heller, 1984) were extensively grazed and little plant material was observed. Most roadsides and native meadows within a few kilometers of a village were closely grazed by goat, sheep, or cattle and collections in these areas were not very productive. This was especially noted in the area north and northwest of Blagoevgrad during the 1993 expedition. Other native meadows were being harvested for hay during the expeditions and a delay of even a few days would have resulted in no collections from those sites. Much of the land in river valleys was intensively utilized in small plots of agricultural and vegetable crops, and few collections could be made at these sites. Utilization of the land for intensive grazing near villages and small plot farming in river valleys greatly increased between the two expeditions. Genetic erosion of the extensive *Trifolium* resources within Bulgaria may occur as these practices increase.

Coniferous forests predominated at elevations greater than 1200–1300 m. Few species other than *T. medium* and *T. alpestre* were collected at these coniferous sites. Mountains and hillsides that were good sites for collection were predominantly limestone- or sandstone-based rather than granite-based. Few *Trifolium* species were observed at granite-based sites throughout both expeditions.

Certain *Trifolium* species were found in very specific environmental niches. The best example in these expeditions was *T. spadiceum*. It was collected almost exclusively growing in the water at the edge of 1 m wide (or less) streams at 1390–1570 m. After discovering this specific environmental niche, we followed small streams at these elevations at several sites and found this species each time. Yet, prior to these expeditions, there were only two accessions of *T. spadiceum* in the NPGS. It is very likely that other rare *Trifolium* species have similar specific environmental niches that have not yet been identified. Identification of the proper microenvironment would increase the probability of collecting these species.

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